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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Robert Taylor (25)
Registration Division (TS-767)

THRU: Orville E. Paynter, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

12.15.82

SUBJECT: Review of Validated Study entitled "Two-Year Chronic Oral Toxicity Study with Dowco 233 In Albino Rats" conducted at IBT (No. 621-06138)
CASWELL#882I

Recommendation:

This study is classified as Core Supplementary Data. There are, however, useful data on body weight, food consumption, organ weights and ratios, hematology, clinical chemistry, urinalysis and neoplasms. It is also concluded in this review that the study has been compromised as a carcinogenic and toxicity evaluation because of incomplete pathology. Consequently a definitive statement relative to oncogenic potential cannot be based nor a NOEL for chronic toxicity be established on data from this study.

Treated females in the mid and high levels tended to weigh less than control females during the second year of the study, but differences were not statistically significant. Heart, liver and kidney body weight ratios for high level females were increased, but the difference was significant only for the heart and kidney. A study of the data for these ratios indicates that the increase is more a reflection of body weight differences between the control and high level females than an increase in absolute organ weights for the high level females.

002342

Review·

Two-Year Chronic Oral Toxicity Study with Dowco 233 in Albino Rats; conducted at IBT (Study No. 621-06138) and submitted by Dow Chemical Co.

(In a validation report, dated 11-19-82, of this study made by Experimental Pathology Laboratories (EPL) the classification of "Supplementary" was assigned because the study "provides data on the histopathological examinations of most tissue masses; in addition, the majority of the study's other biological parameters are supported by raw data". Reported deficiencies of the study were the lack of histological evaluation of tissues with lesions noted during the study and at terminal sacrifice other than those grossly suggestive of neoplasia, and the failure to record daily clinical observations and to report all clinical and gross pathology observations.)

Dowco 233, Ref. GHC-25-1-47, was offered to Charles River albino rats at dietary levels of 3, 10 and 30 mg/kg for two years, beginning January 27, 1975 and terminating January 31, 1977. Groups contained 50 rats/sex and there was a control group offered standard diet. Body weights were recorded initially and at weekly intervals for the first 3 months, then monthly for the remainder of the study. Weight gains were computed at 3, 12 and 24 months and the data statistically analyzed. Food consumption data were collected individually for 10 rats/sex/group weekly during the first 3 months and for 1 week/month for the remainder of the study. Fresh diets were prepared and fed each week. Checks for abnormal reactions and/or deaths were conducted daily with any abnormal reactions and noteworthy observations being recorded weekly for the first 13 weeks, a minimum of once monthly for the 4th through 22nd months and at completion of 24 months. Blood and urine samples were collected individually from 10 rats/sex in the control and high level groups after 3 6 12. 18 and 24 months and from 10 male rats (for clinical chemistry studies only) in the low- and mid-level groups after 24 months of testing. The parameters determined were:

hematology -	WBC	clinical chemistry -	blood glucose
	RBC		BUN
	differential leukocyte		SAP
	Hb		SGPT
	hematocrit		

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urinalysis - glucose
albumin
pH
sp. gravity
microscopic elements

Survivors at 24 months and rats which succumbed during the study, unless precluded by severe postmortem autolyses, were examined grossly and a complete set of organs and tissues (heart. lungs, trachea, liver, spleen, lymph nodes, pancreas, stomach, small and large intestines, kidneys, urinary bladder, pituitary gland, thyroid gland, parathyroid glands, adrenal glands, gonads and associated organs [prostate and uterine horn], brain, spinal cord, peripheral nerve, eyes, optic nerves, salivary glands, skeletal muscle and bone marrow) were preserved. Fixed tissues from 10 rats/sex from control and high level groups surviving 24 months, selected tissues from rats surviving 24 months and selected tissues from rats that were killed while moribund were processed into slides for microscopic examination. Each histopathologic observation judged to be other than normal was recorded and assigned a number grade indicating the degree of severity of change and the incidence and average grade by group and sex were tabulated. In addition to routine examination of tissues, a microscopic examination for evidence of neoplastic change was made on a portion of each grossly observed lesion found in any rat on study. Tabulated neoplastic data included incidence, location, size and pathologic classification. Weights of brain, gonads, heart, kidneys, liver and spleen were recorded and organ-to-body and organ-to-brain weight ratios were calculated; absolute weights and ratios were analyzed statistically.

Results:

Mean male body weights for control and treated rats alike peaked about 16-20 months and showed a relatively consistent decline thereafter; the same pattern was exhibited by treated females but weights for control females increased from a sharp dip shown at 20 months. Treated females in the mid and high levels tended to weigh less than their respective controls during the second year of the study but the differences were not statistically significant ($p < .05$). Mean kidney, heart and liver/body weight ratios of treated females were increased in a dose-related manner, compared to controls, but the differences reached statistical significance only for the kidney and heart of the high level (30 mg/kg). These ratios in gms/100 gms were:

3

	Control	3 mg/kg	10 mg/kg	30 mg/kg
heart	0.3556	0.3682	0.4060	0.4236*
kidney	0.6363	0.6848	0.7173	0.7644*
liver	2.2518	2.2958	2.4710	2.5678

Mean weights (g) of these organs were:

heart	1.586	1.442	1.626	1.599
kidney	2.835	2.681	2.881	2.869
liver	10.088	9.169	10.020	9.795

*Statistically significant difference at the 95% ($p < .05$) confidence level.

There was a sharp divergence between mean body weight for the high level females and the controls near the end of the study (e.g. between months 23 and 24 mean weight for the high level females fell by 18 grams whereas that for controls increased by 21 grams); consequently these ratio changes were more a reflection of body weight changes rather than absolute organ weight increases.

Weights for the lung and a comparison to body weight should have been included in the report.

The EPL validation report noted that clinical observation raw data were sporatic and it was not possible to verify that the rats were checked daily for abnormal reactions and/or death. The report also discussed the presence of numerous clinical and gross pathology observations which were not reported by IBT; these were listed, however, in one of the sponsor's validation reports (prepared by Booz, Allen & Hamilton, Inc.) Most of the clinical observations described the history of lesions and masses. Both lists of unreported data were reviewed and no noteworthy differences were discerned between control and treated rats regarding the unreported clinical observations; there was an increased incidence of unreported gross pathology findings for the liver, spleen and kidney of the low and high level rats, but for each organ the highest incidence was found for the low level. There did not appear to be any bias in failure to report these findings. However loss of this information is crucial to the study since <u>all</u> tissues for which there are gross observations should be examined histologically (See #2 below).

4

The number of survivors at 18 and 24 months was less for both treated males and females (except low-level males at 18 months and mid-level females at 24 months) than for their respective controls. The numbers at these periods were:

	Control 18 mo/24 mo	3 mg/kg 18 mo/24 mc	10 mg/kg 18 mo/24 mo	30 mg/kg 18 mo/24 mo
Males	30/17	31/13	27/13	29/15
Females	37/18	34/16	33/20	31/16

Treated males and females appeared normal when compared to their respective controls for food consumption and hematology, clinical chemistry and urinary parameters. Observed histopathologic alterations did not appear to be related to ingestion of Dowco 233. These conclusions are based upon the data of the study, but the study is compromised in at least three ways, all of which concern histopathological evaluation.

1) The study is compromised as an indicator of carcinogenic potential because a "full" set of tissues was examined histologically only for 10 rats/sex in the control and high level groups. And even this was not accomplished for every tissue, as shown in the sponsor's tabulation of tissues examined in Table 5 of the 2/18/80 validation, where it can be seen that fewer than 10 rats/sex/group were examined for several tissues, e.g. optic nerve, thyroid, parathyroid, cecum and bone marrow. At a minimum, all control and high dose animals must be examined histologically for a study to be considered acceptable (Core-Minimum Data).

2) Sensitivity of the study as an indicator of chronic toxicity was compromised by the policy of histologically examining only those tissues for which lesions were noted during the study or at necropsy which were suggestive of neoplastic change. All tissues for which lesions were noted should have been examined to microscopically fully investigate the toxicological potential of Dowco 233. Furthermore, it is not possible to ascertain with certainty by a gross examination alone whether all findings are neoplastic or not.

002342

Some few samples of tissues for which there were gross comments and which were not examined histologically are:

		Gross Comments
*Animal #334 -	testes	- bilateral decreased size
	kidneys	- bilaterally enlarged
	liver	- enlarged
	lung	- acute pneumonia
	kidney	- cysts
*Animal #242 -	testes	- bilateral decreased size
	kidneys	- yellow foci, bilateral
	lung	- acute pneumonia
	lymph nodes	- peribronchiolar nodes, enlarged cervical lymph nodes, enlarged
	spleen	- enlarged; tan area on spleen
*Animal #228	liver	- numerous dark red depressed areas
	kidneys	- enlarged

*Taken from Appendix 1 of sponsor's report dated 9/17/81 of validation and summation of pathology data for this study.

3) The incidence of pneumonia in the animals of this study was high, possibly a reflection of poor environmental control. Survivorship for males of the mid and high levels was barely over 50% at 18 months, with 27 and 29 rats alive out of 50 for these groups, respectively, and only 30 of 50 male control rats alive at this time. Of the total number of 272 moribund sacrifice and postmortem animals, 217, or 80%, had pulmonary lesions (these figures were taken from EPL validation, attachments G & F). With such a high incidence of pneumonia there is the possibility that more subtle pulmonary changes were overshadowed by infection and consequently lost to the gross examiner at necropsy. Moreover, sensitivity of the study was reduced by the loss of animals due to pulmonary infection.

Core Classification: Supplementary Data

Because of incomplete pathology, this study cannot qualify as core minimum for evaluation of carcinogenic or chronic toxicological potential of Dowco 233, however it provides useful information on growth, organ weights, food consumption, hematology, clinical chemistry, urinarysis and neoplasms in rats exposed to this chemical in their diet.

6

6

A Canadian validation, dated 10/5/81, found the study to be valid. A subsequent memorandum by the same Canadian reviewer, N. Platonow, dated 1/8/82, explained his procedure, which was not specifically mentioned in the earlier audit and validation, for examination of histological and gross pathology data. This study was, however, assigned to the U.S. for validation under the U.S. - Canadian IBT agreement.

The differing opinions between the U.S. (Supplementary) and Canadians (Valid) regarding classification of the IBT study for validation purposes is made inconsequential by an evaluation of the study, which found it to provide only supplementary data for the purpose of supporting registration of the pesticide.

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12/15/82

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